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ACCUMULATION OF BIOGENOUS STIMULATORS IN THE ORGANISM AND ISOLATED TISSUES UNDER THE INFLUENCE OF ULTRAVIOLET RAYS

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The basic aspects of the hypothesis of tissue treatment which were developed by Academician V. P. Filatov as a result of much clinical and laboratory research may be summarized as follows: Animal and plant tissues were excised from the body and observed for biochemical regeneration under the influence of factors which normally hamper life processes. As a result of this regeneration, development of stimulators of life processes takes place in the tissues. These biogenous stimulators, when introduced into an organism, become stimulators in the tissues of the latter. Biogenous stimulators also arise in the entire body that is exposed unfavorably, but not lethally, to a medium through biological regeneration.

Such unfavorable factors of a medium, as is well known, are preservers of animal tissues at low temperatures and of plant tissues in darkness.

According to Filatov, the appearance of biogenous stimulators can be expected under the influence of mechanical, chemical, and thermal factors, and even ultraviolet rays, X-rays, etc., upon the tissue.

I will not discuss the literature on the question of the significance of factors of cooling, darkness, etc., for the development of stimulators in animal and plant tissues. Those who are interested are referred to the works of Filatov and his students.

However, the literature which treats the influence of ultraviolet rays on animal and plant tissues is voluminous. The literature on the medical action

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of ultraviolet rays in a number of diseases of an organism is especially rich. Since it is impossible to present it completely because of the diversity of action of ultraviolet rays, we shall briefly present only what is relevant to the question at hand.

In his monograph, "Optical Transplanting of the Cornea and Tissue Therapy" (1945), Filatov presents data of Kuk and Fardon, which indicates the role of radiant energy in the development of traumatic hormones and "traumatin" as a product of the life activity of the injured cells.

It is known that ultraviolet rays do not penetrate deep into the tissues and are almost completely absorbed by the cells of the epidermis, reaching the surface capillary network and nerve extremities to an insignificant degree. The primary reaction takes place in t[illegible]in, predominantly among the epidermic cells, where significant physicochemical and morphological changes occur (Kaplanskiy, Rotman). In this a significant change in the biochemical processes in the skin takes place, as well as complex changes in its carbohydrate and lipoid metabolism (Kaplanskiy). There are also marked changes in its physicochemical and biological properties which cause a sharp biochemical regeneration of a number of organs and systems.

Thus, a number of authors (Pinkussen, Essinger, and others) indicate the activation of oxidation under the influence of ultraviolet rays.

It was proved by the works of Varshaver, Davydov, and others that work capacity is increased by the effects of ultraviolet radiation, which they connect with an increase in oxidation.

Gese found in the blood of irradiated patients an increased content of albumin which, in his opinion, causes the stimulation in the organs and tissues. This led him to find a similarity between ultraviolet exposure and parenteral albumin therapy.

Radiation in small ultraviolet-ray doses stimulates blood regeneration in severe posthemorrhagic cases and in serious infectious diseases. Large doses of ultraviolet rays, particularly when the dosage is increased too rapidly at excessively frequent intervals, can lead to a decrease in hemoglobin and the number of erythrocytes (Alikin and Varshaver).

Shinkarenko proved in his experiments on rabbits that erythemic doses of ultraviolet radiation cause expansion of free nerve ends in the skin. There is also an expansion of the nerve fibers bearing Merkel's touch cells and branching out into the depth of the epidermis.

Loughborough proved in experiments that, under the influence of irradiation in sublethal doses of ultraviolet rays, yeast began to grow considerably. In this process, substances were deposited containing nitrogen compounds and consisting of decomposition products and nucleinic acid derivatives of the pentonucleotid type.

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In view of these circumstances, and the basic assumptions of the tissue-therapy hypothesis at the suggestion of Filatov, we took up a study of the effect of irradiation on rabbits with ultraviolet rays to produce biogenous stimulators in the isolated tissues and in the general system. We carried out three series of experiments on rabbits for this purpose.

In the first series we studied the effect of irradiation on an isolated portion of rabbit skin in healing a lesion.

For ultraviolet irradiation, we used a Bach portable lamp. The isolated rabbit skin (10 x 10 cm in size) was freed of fur 24 hours before the irradiation.

During irradiation the rabbit was bound to a stand and placed under the Bach lamp in such a way that the shaved portion of the skin (100 sq cm) was directly under the lamp at a focal distance of 30 cm. The exposure time was 10 minutes. The furry parts of the rabbit were covered with a sheet.

Seventy-two hours after irradiation, not only the experimental but also the control rabbits were given lesions in the skin of the anterior ear in the form of a circle 14 mm in diameter. This was done with a trephine, with subsequent separation of the skin with scissors.

Observations on the healing of these wounds and measurements of them in two mutually perpendicular directions were made every day. Six experimental and three control rabbits were under observation.

The observations showed that healing of the affected ear skin in the case of the experimental rabbits was completed in 20-31 days, and in the control rabbits in 32-45 days.

This series of experiments, made on a small number of animals, showed a quicker healing of the skin wound in the irradiated rabbits than in the control.

In the second series of experiments the rate of healing of the skin wound was studied under the influence of a graft under the skin. The graft consisted of a sterilized [The original used the word "autoclaved," here and in all subsequent passages translated "sterilized."] piece of another rabbit's skin, previously subjected to irradiation by ultraviolet rays.

A portion of rabbit skin 100 sq cm was irradiated under a Bach lamp according to the method described above for 10 minutes. This piece was freed of fur 24 hours before the irradiation. Two rabbits, No 166 and No 160, were subjected to such irradiation.

Seventy-two hours after the irradiation the skins of the above-mentioned rabbits were cut in two strips, both 4 x 10 cm in size. One strip was taken directly from the irradiated portion, and the other, from the same rabbit, was taken from an area 7 cm away from the irradiated portion, that is, it was not subjected to the preliminary direct irradiation.

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To prepare an extract, 0.5-gram pieces of skin were taken from the excised portions; an extract was also prepared from the normal skin of a rabbit that had not been irradiated for a control. The rest of the skin, after sterilization, was used the same day for grafting under the skin in the experimental and control rabbits. We prepared five extracts this way (No 1, 2, 3, 4, and 5) in the Laboratory of Conserved Tissues of the Institute, according to Filatov's method, set forth in his instructions on the preparation and use of tissue preparations (Odessa, 1946). Extracts No 1 and No 2 were prepared from the irradiated skins of rabbits No 166 and No 160; No 3 and No 4 were prepared from the nonirradiated skins of the same rabbits, and No 5 was the control.

Grafting under the skin of sterilized irradiated (second series) skin, or skin separated from the irradiated portion (third series) was done in the usual way. After preliminary preparation (removal of hair, painting the skin with 1% brilliant green), a 1.5-sq cm section of skin was taken from the skin on the right side, over the edge of the shoulder blade with scissors, so as to form a pocket. Temporary sutures were made and placed under the skin graft, 4 to 4.5 sq cm, and placed in the pocket. The sutures were tied and the wounds smeared with a 1% solution of brilliant green. Lesions were made in the same rabbits in the skin of the anterior surface of the ear in the form of a circle 14 mm in diameter, and the previously-mentioned epithelization was observed.

Six rabbits (second series) were experimented upon. Sterilized irradiated pieces of skin, from rabbit No 166, were grafted on three of them, and the other three were grafted with sterilized, irradiated pieces of skin from rabbit No 160. Along with the grafting, lesions 14 mm in diameter were made in the skin in the anterior surface of the rabbits' ears.

In addition to these experiments, a third series of observations was made for the purpose of studying the healing rate of analogous lesions in the skin of a rabbit under the effect of grafts of pieces of sterilized skin not subjected to the action of ultraviolet rays. This skin was taken from irradiated rabbits No 160 and No 166.

Six rabbits were experimented upon. Sterilized pieces of skin 4 to 4.5 sq cm (from rabbit No 166) were grafted on three of them, and pieces of sterilized skin of the same size from rabbit No 160 were implanted under the skin of the other three. Along with the grafting, lesions 14 mm in the skin of the anterior surface of the rabbits' ears.

Three rabbits were used as controls for the second and third series of experiments; in these series sterilized normal skin, 4 to 4.5 sq cm, from a nonirradiated rabbit was implanted under the skin of the anterior surface of the ear, and lesions were made as in the case of the experimental animals.

Our observations of the healing process of the skin lesions in the anterior surface of the ear of the experimental and the control rabbits of the second and third series showed that in the rabbits in which directly irradiated skin (second series) was implanted, the healing proceeded very slowly, and by the eighth week complete epithelization was noted in only one rabbit; in the case of the others there were slow granulations over the whole surface.

Quicker healing was observed in the rabbits of the third series than the second, but we also observed slow granulations on the whole affected surface in these cases; epithelization did not appear on the periphery until the fifth week after the beginning of the experiment, and by the eighth week it almost covered two-thirds to four-fifths of the affected surface.

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In the case of the controls, the healing process was slow but regular, and at the end of the observations, that is, by the eighth week, two of them showed complete epithelization; in the third a slight lesion, 3 x 3 mm in size, remained not covered by the epithelium.

Thus, these experiments showed that in the case of the control rabbits the healing processes proceeded considerably faster than in the case of the experimental rabbits. With the rabbits of the second series, it was considerably slower than the third.

To corroborate the results obtained, the extracts prepared from skins No 1, 2, 3, 4, and 5 were tested in the biological and biochemical laboratories of the Institute. In the biochemical laboratory the test of the action of the said skin extracts was performed biologically by Filatov and Docent V. A. Biber.

The action of the extracts in this test was ascertained by their effect on the raising power of the yeast: "This method consists of fermenting a sugar solution of a given concentration with a given quantity of compressed yeast in the presence of the test extract." The fermenting was done in flasks with outlet tubes to collect carbonic acid gas, at 35 to 36 degrees C, while an equivalent amount of physiologic salt solution was added to the control flask, which contained the same amount of sugar and yeast suspension along with the extract. The test took 3 hours. Measurement of the volume of carbonic acid gas given off over each 1½-hour period in the fermentation process gave an idea of the stimulating effect, which can be expressed graphically.

Calculation of volumes of carbonic acid gas given off over the 3-hour period in the test and the control permitted calculating the action of the extract by the formula:

Stimulating activity $(V_1 - V_2) \cdot \varphi$, where V_1 is the volume in milliliters of carbonic acid gas obtained in the test with the extract, V_2 is the volume in milliliters of carbonic acid gas given off in the control flask, and φ is the rarefaction.

The study of the extracts in the above test was made by Biber and L. I. Adamanis, Scientific Worker of the Institute, to whom I am very grateful.

The results of the study are given in Table 1. As can be seen from the table, the skin extract which was directly irradiated with ultraviolet rays turned out to be the least active. The skin extract taken from the radiation area (5.5 - 6.5) proved more active. The normal skin extract approached in its activity the extract of skin removed from the irradiated portion.

These results are in accord with the data obtained in my experiments on rabbits and also with N. V. Yanyk's data (obtained at my request in the biological laboratory of the Institute) on skin extracts as activators of vital activity in yeast. We shall not dwell on the research method, since it is set forth in detail in Yanyk's article, "Morphological Changes in a Yeast Cell Under the Influence of Biogenous Stimulators."

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The experiments showed that the skin extract taken from an irradiated area is less active than the control and the extract prepared from the skin taken from the irradiation site.

In applying the results to our investigations, it is to be emphasized that the small number of rabbits we observed is quite insufficient for the results obtained to be considered conclusive. However, it can now be considered probable that an a skin which has been directly irradiated with ultraviolet rays for 10 minutes, just as in skin which has not been directly irradiated but taken from an irradiated rabbit, there are no biogenous stimulators. This may be explained either by the larger dosage having a depressing effect on the tissue, or by the fact that the period of time (72 hours) was not sufficient to develop biogenous stimulators. The data of the first series of experiments show that stimulating substances were developed in the rabbit's organism under the influence of the irradiation of part of the skin with ultraviolet rays which permitted a quicker recovery of the skin defect in the rabbits than in the control.

In the future it will be necessary to make a series of researches to discover biogenous stimulators in isolated tissues and in the general system with various irradiation doses and also at various times after irradiation.

Table 1

Time	14.1		20.1			21.1		
	Control	Extract No 1	Control	Extract No 2	Extract No 5	Control	Extract No 3	Extract No 4
0.5	9	11	29	28	28	16	15	19
1.0	66	63	83	81	84.5	72.5	75	76
1.5	95	90	119	108.5	114	115	113	113
2.0	98	88	131	127.5	129.5	110	110	102
2.5	124.5	112	106	102.5	106.5	99.5	101	100
3.0	110	114	54.5	51	55	100.5	105	97
Total	510.5	478 -32.5	522.5	498 -24.5	517.5 - 5	513.5	519 +5.5	507 - 6.5

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